PROCEEDINGS
OF THE
CALIFORNIA ACADEMY OF SCIENCES
FOURTH SERIES

Festschrift for George Sprague Myers

Vol. XXXVIII, No. 23, pp. 415–420; 3 figs.; 1 table. December 31, 1970

SIZE AND DISTRIBUTION OF PROTEINS IN ELASMOBRANCH PLASMA

By

Robert J. Heckly

Naval Biomedical Research Laboratory, Naval Supply Center
Oakland, California 94625

and

Earl S. Herald

Steinhart Aquarium, California Academy of Sciences
Golden Gate Park, San Francisco, California 94118

As part of a project sponsored by the Office of Naval Research (N0014-67-C-0343) some members of the staff at Steinhart Aquarium embarked on an electrophoretic investigation of the classification value of elasmobranch plasma proteins. Samples from 11 of the 27 species available were sent to the Naval Biomedical Research Laboratory for ultracentrifuge analysis. The results show species and perhaps family differences not suspected from cellulose acetate and acrylimide gel electrophoresis.

Similarities in blood constituents have been used by many investigators to show relationship between various animals. Genetic changes and natural selection are believed to affect serum or plasma constituents less than the gross anatomy or other such features. Thus, similarities in composition of blood or serum would indicate a relationship; certainly if they differed significantly it would indicate that the individuals are not closely related. Such classification of elasmobranchs has been the subject of immunologic or electrophoretic analyses (Clem

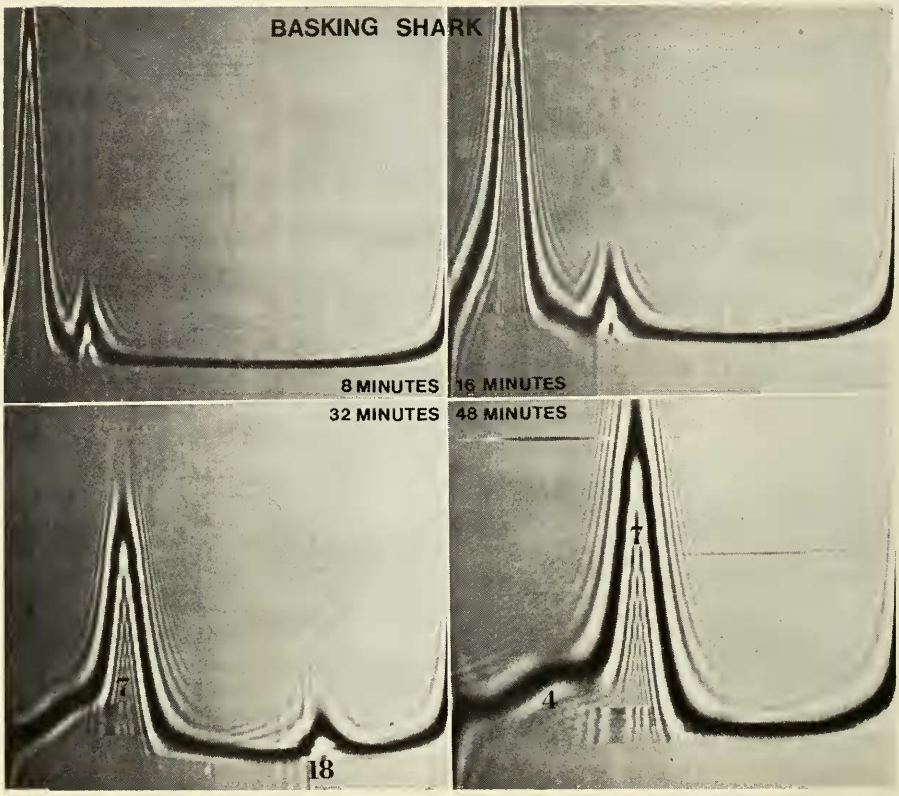

FIGURE 1. Schlieren patterns obtained from plasma sample of 696 cm. adult male basking shark (*Cetorhinus maximus*). Photographs were taken at indicated times after rotor attained full speed (59,780 rpm). Numbers below major peaks in last two frames refer to observed sedimentation coefficients.

and Small, 1967; Irisawa and Irisawa, 1954; Rasmussen and Rasmussen, 1967; Shuster and Goodman, 1968) but no comparative study of molecular size of serum proteins has been made. This report describes the results of some ultracentrifugal analyses of blood plasma from several selected species.

MATERIALS AND METHODS

Blood was usually obtained by caudal puncture using Sequester-Sol (a dipotassiumethylenediaminetetracetate supplied by Cambridge Chemical Products, Inc., Detroit) and for most samples, immediately centrifuged; the plasma was then removed and stored at 2° C. Analyses were made with a Spinco Model E Analytical ultracentrifuge fitted with schlieren optics using 12 mm. cells. The plasma were analyzed at the highest practical concentrations, either 1:2 or 1:4,

TABLE 1. *Sedimentation coefficients of the principal plasma proteins.*

Common Name	Scientific Name	Sex	CM Total Length	Total Protein	Component S Values			
Pacific lamprey,	*Lampetra tridentata*	F	49.5	3.1	3.4	8	13	—
Sevengill,	*Notorynchus maculatus*	F	64.7	1.9	3	7	—	17
Horn shark,	*Heterodontus francisci*	M	75.7	5.3	4	7	—	17
Basking shark,	*Cetorhinus maximus*	M	696.0	—	4	7	—	18
Swell shark,	*Cephaloscyllium ventriosum*	F	94.0	3.4	4.5	7	—	17
Leopard shark,	*Triakis semifasciata*	F	119.3	2.75	4.3	7	14	17
Dogfish,	*Squalus acanthias suckleyi*	F	—	—	4	7	—	17
Shovelnose guitarfish,	*Rhinobatos productus*	F	89.0	5.9	5.6	8	12	17
Thornback ray,	*Platyrhinoidis triseriata*	F	57.2	0.64	4	8	—	17
Pacific electric ray,	*Torpedo californica*	M	78.6	—	5	8	12	17
Big skate,	*Raja binoculata* (A)	M	76.2	—	5	11	14	—
Big skate,	*Raja binoculata* (B)	M	90.2	—	5	11	14	—

so that minor constituents would be detected. In all instances the centrifuge was operated at 59,780 rpm.

RESULTS

Figure 1 shows a series of photographs taken during the sedimentation of serum from a basking shark. The first three frames clearly show the separation and sedimentation of the 18 S component. However, even after 48 minutes the 4 S component had not been completely resolved from the 7 S proteins. Since the area under the curve is proportional to the concentration of that component it is clear that the major protein in this serum had a high molecular weight. If the 7 S material is a globular protein, its molecular weight is probably in excess of 160,000.

The results of ultracentrifuge analyses of plasma from a number of animals are summarized in figures 2 and 3 and the observed sedimentation coefficients are listed in table 1. The relative concentrations of the various components in each plasma can be estimated from the schlieren patterns.

Except for the sevengill shark and thornback ray, the relative concentration of macroglobulin (17 S component) in shark plasma is higher than in mammalian serum. As is evident in figure 2, the 7 S globulins in human serum have not been resolved from the albumin, but of course, continued centrifugation did separate the 7 S proteins from the albumin. Similarly, low molecular weight proteins were resolved from the 6 to 8 S components in the other plasma on prolonged centrifugation indicating that there is at least a small amount of albumin-sized material in all of the elasmobranch plasma, even though this is not evident in the 32-minute frames shown in figures 2 and 3.

In addition to indicating relative concentration, the schlieren patterns provide a measure of homogeneity of the protein components in that if all mol-

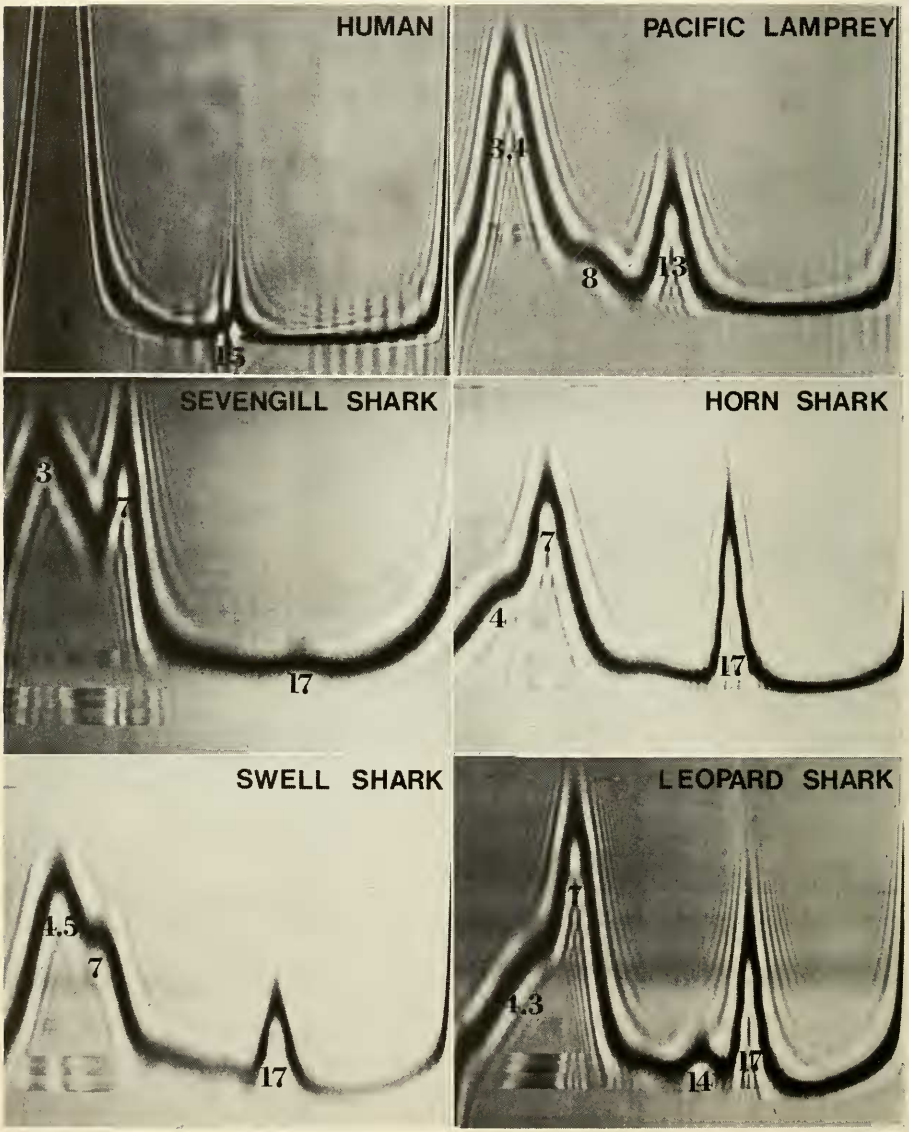

FIGURE 2. Schlieren patterns obtained with various plasma 32 minutes after rotor attained full speed, 59,780 rpm. Numbers given below patterns indicate approximate sedimentation coefficients of components represented by peaks.

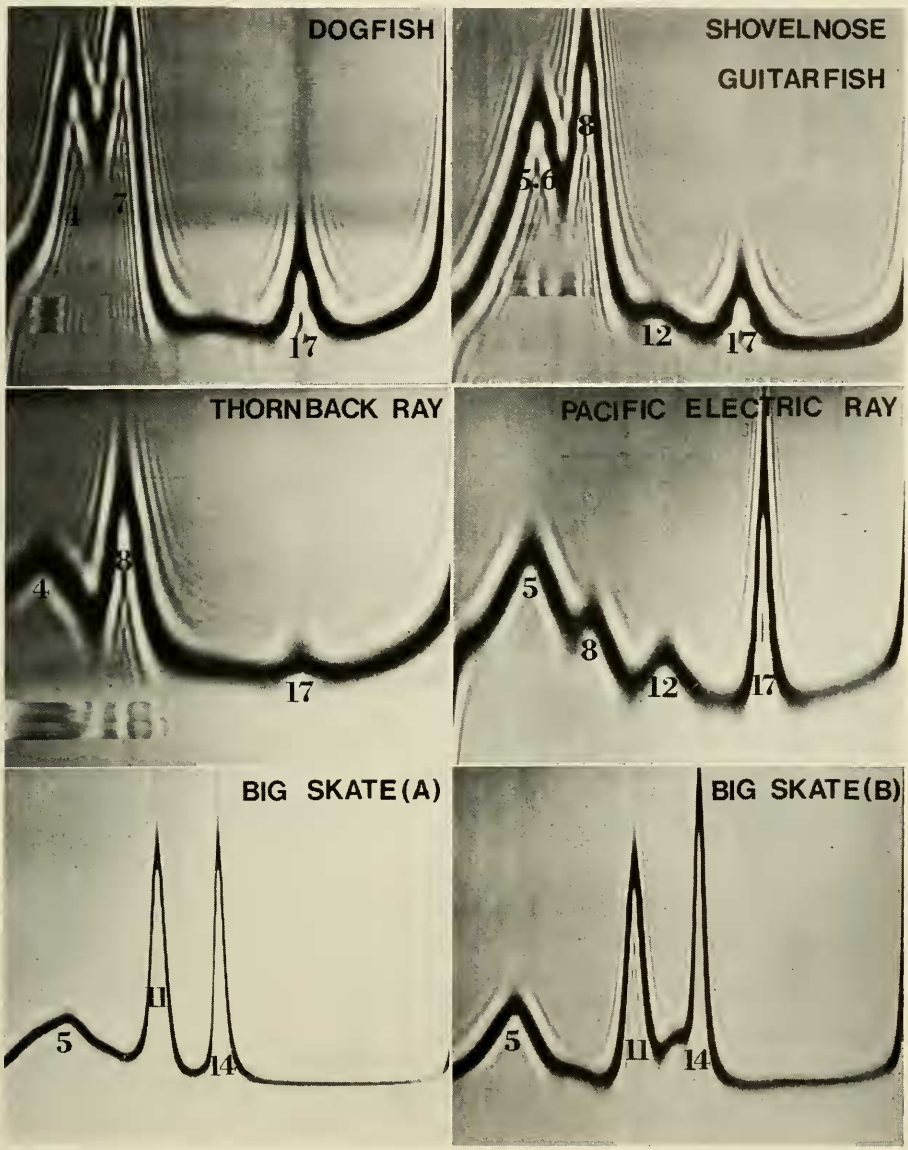

FIGURE 3. Schlieren patterns obtained with various plasma 32 minutes after rotor attained full speed, 59,780 rpm. Numbers given below patterns indicate approximate sedimentation coefficients of components represented by peaks.

ecules are the same size the schlieren peak will be sharp, such as is demonstrated by the patterns for the skate and ray. A broad peak indicates either heterogeneity of the component with respect to sedimentation velocity or a high diffusion coefficient. For instance, albumin, even though it is homogenous, would result in a rather broad peak after 32 minutes.

The lamprey is unusual in that it appears to have a higher relative concentration of albumin (the 3.4 S component) than the elasmobranchs, and in this respect it is more like mammalian serum because, as is well known, albumin is the principal constituent in normal mammalian serum. In contrast, as is shown in figure 1, the principal protein in the basking shark was of the 7 S variety.

Skates appear to lack 17 S protein in their plasma but they have high concentrations of both the 10 and 14 S proteins. Although the patterns from different skates do not yield identical patterns the variations that have been observed are shown in the last two photographs of figure 2. Since the presence or absence of a component is perhaps more significant than small differences in concentration, it is of interest that neither the lamprey nor the big skate have 17 S proteins and that they both have proteins in the 8 to 14 S range.

Also of interest are the differences in the component S values between the guitarfish and the thornback. These two species were formerly placed in separate families now combined in a single family (Rhinobatidae). It would seem logical that all family members would have similar sedimentation coefficients, yet in this case the shovelnose has a 12 S component which is strangely lacking in the thornback. Further study will be needed to evaluate this difference.

As is shown in figures 2 and 3 and in table 1, the leopard shark, shovelnose, guitarfish, and electric ray have four distinct S components in their plasma, whereas all the other eight species studied have only three major components. With the exception of the guitarfish and thornback all species are representatives of individual families not closely related.

LITERATURE CITED

CLEM, L. W., AND P. A. SMALL

1967. Phylogeny of immunoglobulin structure and function. I. Immunoglobulins of the lemon shark. Journal of Experimental Medicine, vol. 125, pp. 893–920.

IRISAWA, H., AND A. F. IRISAWA

1954. Blood serum protein of the marine Elasmobranchii. Science, vol. 120, pp. 849–851.

RASMUSSEN, L. E., AND R. A. RASMUSSEN

1967. Comparative protein and enzyme profiles of the cerebrospinal fluid, extradural fluid, nervous tissue and sera of elasmobranchs. Chapt. 25, pp. 361–379. *In* Perry W. Gilbert (Ed.) Sharks, skates and rays. Baltimore, Johns Hopkins Press, 624 pp.

SHUSTER, J., AND J. W. GOODMAN

1968. Phylogenetic studies of shark immunoglobulins. Nature, vol. 219, pp. 298–299.